COMPARISON OF THE PERFORMANCE OF THE IDEXX SEDIVUE DX® WITH MANUAL MICROSCOPY FOR DETECTION OF CELLS AND CRYSTALS IN URINE SEDIMENTS

A Thesis

by

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ABSTRACT

Microscopic evaluation of urine sediments is underutilized in veterinary clinics. The IDEXX SediVue Dx® Urine Sediment Analyzer (SediVue) has been recently introduced for automated analysis of canine and feline urine. The objective of this study was to compare the performance of the SediVue with manual microscopy for detecting clinically significant numbers of cells and crystals.

Five hundred thirty-four urine samples (81% canine, 19% feline) were evaluated. For SediVue analysis (1.0.0.0 and 1.0.1.3), 165 μL of well-mixed, uncentrifuged urine was pipetted into a disposable cartridge. Seventy high-resolution images were captured and processed using a veterinary-specific neural network algorithm. For manual microscopy, urine was centrifuged to obtain sediment. Red blood cells (RBC), white blood cells (WBC), squamous epithelial cells (EPI), and non-squamous epithelial cells (NEC) were quantified by averaging the number of cells in 10 high power fields (HPF), while struvite crystals (TRI) and calcium oxalate dihydrate crystals (CaOxd) were placed into a semi-quantitative category. For clinical significance, a threshold of ≥ 5/HPF was used for RBC and WBC, while a threshold of ≥ 1/HPF was used for epithelial cells and crystals. Using these thresholds, sensitivity and specificity of the SediVue for formed element detection were determined as compared to manual microscopy.

The sensitivity of the SediVue (1.0.1.3) was good for detection of RBC, WBC, and TRI; moderate for CaOxd; fair for NEC; and poor for EPI. Specificity was excellent for EPI and CaOxd; good for RBC, WBC, and NEC; and moderate for TRI. The newer

software version (1.0.1.3) displayed improved sensitivity but decreased specificity for most elements compared to the older version (1.0.0.0). Performance of the Sedivue was overall similar for canine versus feline samples as well as between fresh and stored samples. Precision of the instrument was acceptable.

The SediVue Dx provides diagnostically useful information regarding the presence of clinically significant numbers of formed elements in canine and feline urine and should increase the number of complete urinalyses performed in private practice. Performance is best for RBC, WBC, TRI, and CaOxd, while improvement is needed for epithelial cells. Further software development should continue to enhance accuracy of formed element detection.

DEDICATION

This thesis is dedicated to my parents and fiancé for all of their encouragement and support throughout my residency and master's program.

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This work was supervised by a thesis committee consisting of Dr. Mary Nabity (advisor) and Dr. Karen Russell of the Department of Veterinary Pathobiology and Dr. Johanna Heseltine of the Department of Veterinary Small Animal Clinical Sciences.

Statistical analyses were performed by Jeremy Hammond and Suzanne Edwards of IDEXX Laboratories. All other work conducted for the thesis was completed by Dr. Hernandez with laboratory assistance from Dr. Alex Myers, as well as the technicians and technologists employed in the Texas A&M University Veterinary Clinical Pathology Laboratory and the IDEXX Reference Laboratory (North Grafton, MA).

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NOMENCLATURE

AIM	Automated intelligent microscopy
HPF	High power field
RBC	Red blood cells
WBC	White blood cells
UTI	Urinary tract infection
APR	Automated particle recognition
EPI	Squamous epithelial cells
NEC	Non-squamous epithelial cells
TRI	Struvite crystals
CaOxd	Calcium oxalate dihydrate crystals
LPF	Low power field
ROC	Receiver operating characteristic
CV	Coefficient of variation
QCM	Quality control material

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1. INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Urinalysis is a key component of the evaluation of the urinary tract in patients with urologic disease. Urinalysis is also an important part of the minimum laboratory database and should be included in the diagnostic work-up of all veterinary patients, as it may provide valuable information, even in patients without overt signs of urinary tract disease (e.g., measurement of urine specific gravity to classify azotemia; detection of glucosuria or ketonuria in diabetic patients; identification of occult urinary tract infections). A complete urinalysis consists of macroscopic examination (color, turbidity, and urine specific gravity), biochemical (e.g., dipstick) analysis, and microscopic urine sediment examination. Of these, sediment examination is the component of the complete urinalysis that presents the biggest challenge for veterinarians and veterinary technicians. Microscopic urine sediment examination is time-consuming and associated with high inter-observer variability.[1-3] In addition, in the authors' experience, many veterinarians and veterinary technicians have limited training in urine sediment analysis. Unfamiliarity with identification of the various formed elements in urine, as well as the adjustments that must be made to the microscope for effective examination of urine sediments (e.g., adjusting the condenser and focus), are a few of the reasons why urine sediment evaluation may be intimidating to veterinary personnel and may not be performed in private practice.

1.2 Yellow IRIS™ Urinalysis Workstation

In human medicine, several instruments have been introduced in an attempt to automate urine sediment analysis. The first of these instruments was released in 1983 by Iris Diagnostics as a component of the Yellow IRIS™ urinalysis workstation. This workstation incorporated 3 analytic subsystems for complete automation of urinalysis: the slideless microscope with Automated Intelligent Microscopy (AIM), the IRIS MGM mass gravity meter for specific gravity measurement, and the Ames Clinitek dipstick chemistry reader.[2] The slideless microscope utilized a built-in camera to capture images of formed elements in urine sediments, which were analyzed using image-recognition technology to identify and quantify cells, crystals, casts, and other structures.[4] The introduction of the Yellow IRIS™ urinalysis workstation laid the foundation for the development of numerous other instruments for automated sediment analysis. Although a number of instruments have been developed for this purpose, the most widely used instruments in human laboratories over the past 20 years include the UF-100™, iQ®200, and UriSed.

1.3 UF Series

The UF-100™ (Sysmex Corporation, Kobe, Japan), introduced in 1998, utilizes a combination of impedance technology and flow cytometry to count and identify formed elements in urine samples. The UF-100™ requires 800 μL of uncentrifuged urine for analysis. Once the sample is aspirated into the system, two fluorescent dyes are added: phenanthridine, which stains nucleic acids, and carbocyanine, which stains cell

membranes, nuclear membranes, and mitochondria. After staining, the sample passes through an argon laser light beam.[5] For each element, electrical impedance data are used to measure volume, while forward light-scatter is used to measure size. In addition, the pattern of fluorescence is used to assess nuclear and cytoplasmic characteristics. A combination of size, shape, volume, and staining characteristics is utilized for identification of formed elements.[6] Formed elements identified and quantified per μL or high power field (HPF) by the UF-100™ include red blood cells (RBC), white blood cells (WBC), squamous epithelial cells, hyaline casts, and bacteria. For several other elements, a quantitative result is not reported, but their suspected presence is flagged by the instrument. These elements include small round cells (i.e., tubular and transitional epithelial cells), pathological (i.e., non-hyaline) casts, crystals, spermatozoa, and yeast.[5,7]

The UF-100™ demonstrates good agreement with manual microscopy for detection of RBC, WBC, and squamous epithelial cells.[6,8] Interestingly, one study found that the UF-100™ detected higher numbers of RBC, WBC, and squamous epithelial cells compared to manual microscopic examination. The authors of this study considered the UF-100™ to be the more reliable of the two methods, postulating that manual microscopy had underestimated the number of elements due to cellular destruction during centrifugation or other steps of sediment processing. However, no attempts were made to substantiate this claim.[6] In another study, the UF-100™ was found to overestimate RBC in comparison to manual microscopy. However, in this case, the authors suspected that the presence of bacteria, yeast, and crystals had interfered with RBC detection by the UF-100™, causing false positive results for RBC.[8] In contrast to

the previously discussed elements, detection of casts by the UF-100™ is consistently poor.[5,6,8,9] In addition, performance is overall unsatisfactory for detection of yeast, crystals, and small round cells.[5,9]

Studies assessing the ability of the UF-100™ to accurately detect bacteria have conflicting results. In one study, in comparison to bacterial culture, the UF-100™ displayed a sensitivity of 87% and a negative predictive value of 95% for detection of bacteria using a cut-off value of > 1800/μL. Specificity and positive predictive value (80% and 56%, respectively) were lower, potentially due to interference from crystals or other material. Using a combination of bacterial results with a WBC count of > 45/μL resulted in a substantial improvement in specificity and positive predictive value for the diagnosis of urinary tract infections (UTIs) (95% and 86%, respectively).[8] However, in another study, dipstick results for leukocyte esterase and nitrites in combination with UF-100™ analysis for bacteria and WBC (using cut-off values of 1000/μL and 20/μL, respectively) did not accurately predict the outcome of bacterial culture.[10] Importantly, the UF-100™ cannot distinguish between live and dead bacteria.[6] This is a limitation for all instruments that perform automated sediment analysis, as well as for manual microscopy. Additional pitfalls regarding the identification of bacteria in urine sediments by manual microscopy include the high threshold necessary for reliable detection of bacteria (> 10,000 bacteria/μL for rods and 100,000 bacteria/μL for cocci) and difficulties in distinguishing amorphous crystals from bacterial cocci.[11] Therefore, bacterial culture remains the gold standard for diagnosis of UTIs.[6]

The successor of the UF-100™, the UF-1000i™, was released in 2006. Like the UF-100™, the UF-1000i™ demonstrates good agreement with manual microscopy for detection of RBC, WBC, and squamous epithelial cells. For casts, sensitivity and negative predictive value were reported to be 95% and 99%, respectively; however, specificity and positive predictive values were lower (81% and 33%, respectively). Therefore, verification by manual microscopic examination is required for samples with positive results for casts.[12] The most significant update for the UF-1000i™ was the introduction of 2 separate counting channels: one for cells, casts, and crystals, and the other for microbes (i.e., bacteria and yeast). This update was intended to improve detection of bacteria, while also reducing the interference of bacteria and yeast with RBC quantification.

Various studies have evaluated the use of the UF-1000i™ as a screening tool for UTIs. In most of these studies, specific cutoff values for WBC, bacteria, or a combination of WBC and bacteria provided sufficiently high sensitivity to support the use of the UF-1000i™ as a screening test, reducing the number of samples submitted for bacterial culture by as much as 55%.[13-15] However, the authors of another study concluded that, for their patient population, the reduction in workload and costs associated with bacterial culture did not justify use of the UF-1000i™ as a screening tool, as there was an unacceptably high number of false-negative results.[16] This discrepancy is likely due to the fact that the studies used different definitions for a negative culture result; while the former studies defined a negative culture as $< 10^5$ CFU/mL or $< 10^4$ CFU/mL, the latter study defined a negative culture as no bacterial growth.[13-16] In a

systematic review and meta-analysis of the UF-100™ and UF-1000i™ for UTI screening, the authors confirmed that these instruments show promise as effective screening tools for UTIs. However, none of the studies evaluated in the systematic review used signs or symptoms of UTI as inclusion or exclusion criteria. Therefore, the study populations may not have been reflective of the population of patients in which UTI would be suspected, thereby limiting the clinical applicability of the results. Ultimately, the authors concluded that more vigorous studies are needed to critically assess the utility of flow cytometric analysis of urine for UTI screening.[17]

The most recent addition to the UF series of flow cytometers is the UF-5000/4000, released in 2015. The UF-5000/4000 is reported to exhibit improved detection of bacteria (including discrimination between gram-positive and gram-negative organisms) and enhanced differentiation between RBC and crystals. In addition, the UF-5000/4000 may be used for detection of RBC, WBC, and bacteria in body fluids (manufacturer's website). No peer-reviewed articles in English are available regarding the performance of this instrument.

1.4 iQ®200

The iQ®200 (Iris Diagnostics, Chatsworth, CA, USA), introduced in 2003, is another instrument available for automated human urine sediment analysis. The iQ®200 uses similar technology to its predecessor, the Yellow IRIS™ slideless microscope. For iQ®200 analysis, a minimum of 3 mL of uncentrifuged urine is required. One mL of urine is aspirated by the instrument, and 2 μL are utilized for analysis. The urine is

hydro-dynamically focused in a planar flow cell, allowing a camera-microscope system to capture 500 frames of the urine sediment. Within each frame, images of individual particles are isolated. The particle images are then analyzed by an automated particle recognition software (APR™), which identifies each particle based on size, shape, contrast, and texture. There are 12 particle categories: RBC, WBC, WBC clumps, squamous epithelial cells, non-squamous epithelial cells, hyaline casts, pathological casts, bacteria, yeast, crystals, mucus, and sperm. Quantitative results are reported for each particle type and may be expressed per μL or per high/low power field.[18-20] On the user interface, the operator is able to choose a specific particle category and simultaneously view all elements placed into that category by the instrument. The operator also has the ability to re-classify images before finalization of results.[19]

In one of the earliest studies evaluating the iQ®200, the instrument exhibited statistically significant correlations with manual microscopy for detection of RBC, WBC, and squamous epithelial cells ($\rho = 0.68$, 0.60, and 0.66, respectively).[18] In the same study, the iQ®200 showed 97% sensitivity and 79% specificity for detection of abnormal urine sediments; however, an abnormal sediment was defined as containing > 5 RBC/HPF *or* > 5 WBC/HPF on manual microscopy, and > 17 RBC/μL *or* > 29 WBC/μL on iQ®200 analysis.[18] While these results suggest that the iQ®200 may be a valuable screening test for detection of sediments with clinically significant hematuria or pyuria, they do not characterize the sensitivity and specificity for RBC and WBC as individual elements. In a later study comparing the performance of the iQ®200 to manual phase contrast microscopy, the iQ®200 displayed acceptable sensitivity and specificity

for detection of RBC (73% and 88%), WBC (84% and 93%), and squamous epithelial cells (77% and 99%) compared to phase contrast microscopy. Performance for non-squamous epithelial cells, casts, crystals, bacteria, and yeast was less reliable.[19]

Two studies have assessed the value of iQ®200 analysis as a screening method for UTIs. Using a combination of results for WBC and bacteria at specific cutoffs, the iQ®200 showed moderate to high sensitivity (88% and 95%) for detection of UTIs in comparison to bacterial culture, while specificity was low (66% and 61%).[21,22] Interestingly, in one of these studies, the sensitivity markedly increased from 88% to 98% when "expert review" was used as the gold standard for diagnosis of UTI in place of bacterial culture (where "expert review" considered the presence of urinary tract symptoms and the clinician's decision to treat with antibiotics, in addition to bacterial culture results).[21] Application of these cutoffs was predicted to result in reduction of urine cultures by 55% in one study and 35% in the other, suggesting that iQ®200 analysis could be valuable to significantly reduce cost and labor associated with this procedure.[21,22]

1.5 UriSed

Introduced in 2008, another option for automated sediment analysis in human medicine is the UriSed (77 Elektronika, Budapest, Hungary), also known as the sediMAX® in some countries. For its analysis, the UriSed requires 2 mL of uncentrifuged urine, 200 μL of which is automatically pipetted into a disposable cuvette. The sample is centrifuged for 10 seconds, and 15 high-resolution images of the sediment

are captured using a built-in bright-field microscope and camera. The images are evaluated by a neural-network-based image-processing algorithm (i.e., image recognition software) to identify formed elements. In this way, the technology used by the UriSed is similar to that of the iQ®200. The major difference between the two instruments is that, while the iQ®200 captures images of individual formed elements, the UriSed captures images of an entire microscopic field, similar in magnification to a traditional HPF of 400X magnification.[20,23,24] Elements identified by the UriSed include RBC, WBC, squamous epithelial cells, non-squamous epithelial cells, hyaline casts, pathological casts, calcium oxalate monohydrate crystals, calcium oxalate dihydrate crystals, struvite crystals, uric acid crystals, bacteria, yeast, sperm, and mucus. Results can be reported quantitatively (e.g., per μL, high power field, or low power field), or on a semiquantitative scale (e.g., 1+, 2+, or 3+), depending on the element.[23] Also similar to the iQ®200, images may be reviewed by the operator to re-classify elements that may have been incorrectly identified by the instrument.[20,23,24] An updated version of this instrument, called the sediMAX conTRUST®, utilizes both bright-field and phase-contrast microscopy to capture images of urine sediments.[25]

In one study, the UriSed exhibited the following sensitivities and specificities for formed element detection: 75% and 99% for RBC, 72% and 98% for WBC, 52% and 69% for hyaline casts, 54% and 82% for pathological casts, 100% and 100% for crystals, and 69% and 85% for bacteria. However, the study did not report the number of samples that were positive on manual microscopy for each element, making these results somewhat difficult to interpret.[24] In another study, the performance of the UriSed for

detection of RBC, WBC, casts, and bacteria was similar to that previously described above.[23] In addition, the diagnostic performance of the UriSed was evaluated for several other elements, with the following sensitivities and specificities: 74% and 87% for yeast, 82% and 86% for calcium oxalate crystals, 86% and 93% for squamous epithelial cells, and 62% and 91% for non-squamous epithelial cells.[23]

As with the UF-100™ and iQ®200, multiple studies have evaluated the utility of the UriSed as a screening method for UTIs. Two of these studies demonstrated excellent sensitivity (> 95%) of the UriSed for detection of UTIs using a combination of WBC and bacteria, with bacterial culture as the gold standard.[26,27] However, another study contradicted these results, reporting much lower sensitivity (ranging from 19.1-73.5%, depending on the cutoff-values used) for detection of UTIs, utilizing a combination of UriSed counts for bacteria and WBC, as well as dipstick results for leukocyte esterase and nitrites.[28] These conflicting results could be due to the fact that the studies used different gold standards for the definition of a positive bacterial culture (10^4 CFB/L versus 10^4 CFU/mL versus 10^5 CFU/mL), as well as different cut-off values for numbers of WBC and bacteria to define a positive UriSed result.

1.6 Advantages of Automated Sediment Analysis

Automated urine sediment analyzers provide numerous advantages over manual microscopy. The UF-100™/1000i™, iQ®200, and UriSed display greater intra-assay precision than manual microscopic examination for detection of formed elements.[8,20,29-31] Additionally, automated urine sediment analysis greatly enhances turnaround time for

urinalysis results. The reported number of samples that can be processed per hour by these analyzers are as follows: 86-100 samples/hour for the UF-100™, 60-65 samples/hour for the iQ®200, and 62-100 samples/hour for the UriSed.[18,23,24,30] Lastly, a combination of automated sediment analysis and dipstick results has been utilized in human laboratories as a screening method to reduce the number of samples that require manual microscopic review.[5,7,8,23,25,32,33] However, these instruments do not completely eliminate the need for manual microscopy, as pathological samples are often flagged for technician review.[20,25,33]

1.7 IDEXX SediVue Dx® Urine Sediment Analyzer

The recently introduced IDEXX SediVue Dx® Urine Sediment Analyzer (IDEXX Laboratories, Westbrook, ME, USA) is the first instrument modified to perform automated urine sediment analysis for veterinary patients. This instrument is closely modeled after the UriSed, but the image-recognition software has been adapted for use in veterinary species. The SediVue utilizes 165 μL of uncentrifuged urine to capture high magnification images of urine sediments. These images are analyzed using a built-in veterinary-specific neural-network algorithm, which allows for identification and quantification of formed elements. IDEXX Laboratories is currently continuously updating the neural-network algorithm to improve detection of formed elements. The automation of urine sediment analysis should decrease the amount of time necessary for sediment examination, improve precision of formed element identification, and increase the number of complete urinalyses that are performed in private practice. The objective

of this study was to compare the performance of the SediVue with the reference method of manual microscopy for detection of clinically significant numbers of red blood cells, white blood cells, epithelial cells, and crystals in canine and feline urine sediments.

2. MATERIALS AND METHODS

2.1 Urine Samples

Leftover urine samples were obtained from client-owned canine and feline patients presenting to the Texas A&M University Veterinary Medical Teaching Hospital (n=263) between August 2015 and February 2017. Additionally, leftover canine and feline urine samples submitted to the IDEXX Reference Laboratory in North Grafton, MA (n=298) and the Texas A&M Veterinary Medical Diagnostic Laboratory (n=1) were included in the study. For study inclusion, a total volume of > 1.0 mL was required for centrifugation to obtain sediment for manual microscopy, in addition to a small amount of uncentrifuged urine (approximately 200 μL) for SediVue analysis. Urine samples from healthy patients, as well as patients with urologic or non-urologic disease, were permitted. Multiple samples from the same patient were allowed, as long as greater than 12 hours had passed between subsequent samples. Each sample was evaluated by the SediVue (software versions 1.0.0.0 and 1.0.1.3) and by manual microscopy.

2.2 SediVue Analysis

For SediVue analysis, 165 μL of well-mixed, uncentrifuged urine was manually pipetted into a disposable cartridge. After a 10-second centrifugation period, 70 high-resolution, grayscale images of the sediment were captured using a built-in camera-microscope system (Figure 1). Together, these images covered an area equivalent to approximately 45 HPF. The images were analyzed by the instrument using a veterinary

specific neural-network algorithm to identify and quantify formed elements. Formed elements identified by the SediVue included: red blood cells (RBC), white blood cells (WBC), squamous epithelial cells (EPI), non-squamous epithelial cells (NEC), struvite crystals (TRI), calcium oxalate dihydrate crystals (CaOxd), and unclassified crystals (CRYu, i.e., crystals other than TRI or CaOxd). Casts and bacteria were also identified and quantified, but were not included for analysis in this study. The SediVue provided both semi-quantitative and quantitative results for each element. For semi-quantitative results, each element was placed into one of several possible semi-quantitative categories. The semi-quantitative categories varied depending on the element (Table 1). For quantitative results, the average number of each element identified per HPF or low power field (LPF), when appropriate, was reported. Once collected, images could be re-analyzed with multiple iterations of the neural-network algorithm to detect changes in performance between software versions. Results from software versions 1.0.0.0 and 1.0.1.3 were used for this study.

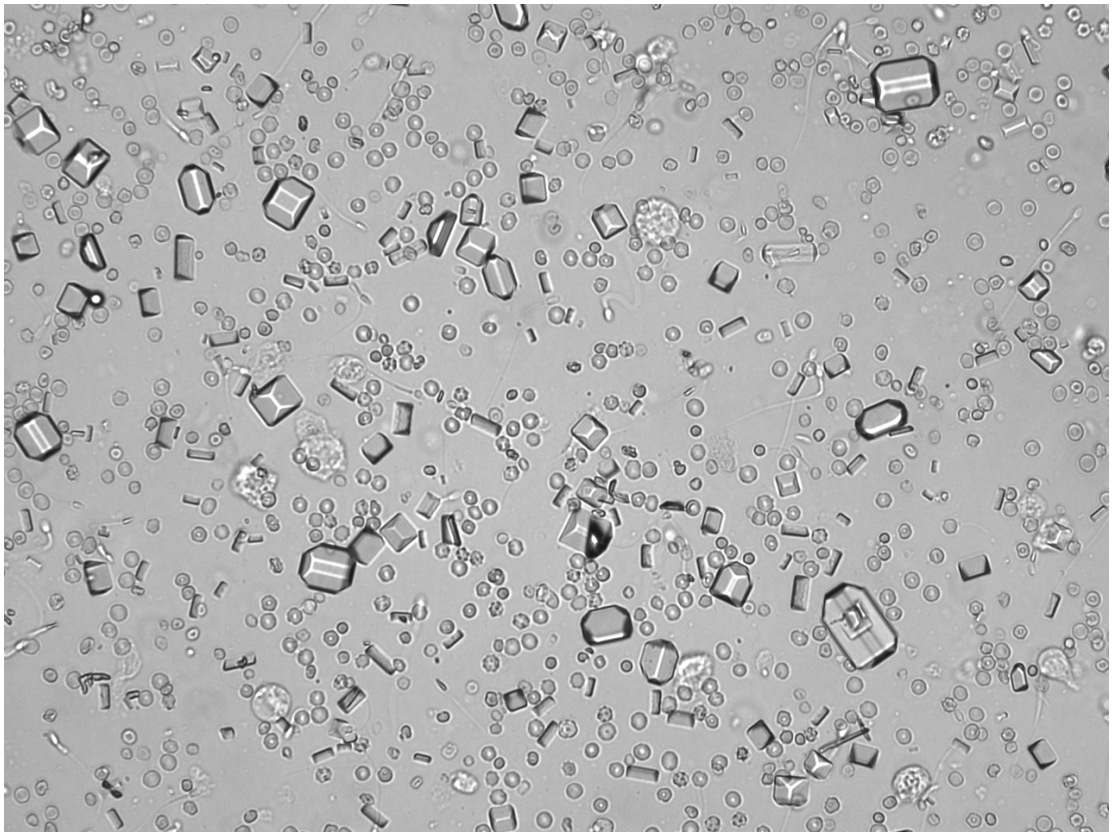

Figure 1. Example of an image taken by the SediVue. Each image represents approximately 66% of a typical microscopic 400X field.

Table 1. Semi-quantitative categories used for each element.

Element	Categories				
RBC	None to rare	1-5/HPF	6-20/HPF	21-50/HPF	>50/HPF
WBC	None to rare	1-5/HPF	6-20/HPF	21-50/HPF	>50/HPF
EPI	None to rare	1-2/HPF	3-5/HPF	6-10/HPF	>10/HPF
NEC	None to rare	1-2/HPF	3-5/HPF	6-10/HPF	>10/HPF
TRI	None to rare	1-5/HPF	6-20/HPF	21-50/HPF	>50/HPF
CaOxd	None to rare	1-5/HPF	6-20/HPF	21-50/HPF	>50/HPF

RBC, red blood cells; WBC, white blood cells; EPI, squamous epithelial cells; NEC, non-squamous epithelial cells; TRI, struvite crystals; CaOxd, calcium oxalate dihydrate crystals; HPF, high power field.

2.3 Manual Microscopic Examination

Within one hour of SediVue analysis, each sample was reviewed by manual bright-field microscopy. A minimum of 1.1 mL (range 1.1-4.2 mL) of urine was centrifuged at 1,228 x *g* for 5 minutes. The sediment was processed using a KOVA® system[a] according to manufacturer's instructions and loaded into a DeciSlide™[b]. The same DeciSlide™ preparation was reviewed separately by two individuals skilled in interpretation of urine sediments from a group of clinical pathology residents, clinical pathologists, and laboratory personnel (veterinary technicians and medical technologists). A maximum of 1 hour was allowed between first and second review, and both observers were blinded to the results of SediVue analysis. Upon review of the urine sediment, one observer assigned each element to a semi-quantitative category. The other observer reviewed the same urine sediment preparation and independently placed each formed element into a semi-quantitative category. If there were any discrepancies between the two observers with regards to the most appropriate semi-quantitative category for each element, the observers attempted to come to an agreement. If an agreement could not be reached, a third observer was consulted in order to reach a majority decision. In addition to the semi-quantitative assessment, one observer performed a quantitative assessment by counting the number of RBC, WBC, EPI, and NEC per 10 HPF. The average number of each element per HPF was recorded. When necessary, acid or base was added to the urine sediment to aid in determination of crystal

[a] KOVA® Tubes and KOVA® Petters, KOVA International Inc., Garden Grove, CA
[b] Fisherbrand™ UriSystem™ DeciSlide™ 10-Test Slides. Fisher Healthcare, Houston, TX

types, as different types of crystals display characteristic solubility profiles upon the addition of acidic or basic compounds. For documentation, one observer captured a minimum of 5 representative images of each urine sediment using a digital microscopy camera.[c]

2.4 Determination of Intra- and Inter-assay Precision for RBC and WBC

The intra- and inter-assay precision of the SediVue for detection of RBC and WBC were assessed using commercial quality control material (QCM).[d] For intra-assay precision, 2 levels of QCM (normal and abnormal) were analyzed 10 consecutive times. For inter-assay precision, 2 levels of QCM were analyzed once daily for 5 days.

Intra-assay precision was also assessed using fresh canine and feline urine samples (leftover from clinical specimens). One to six samples were included for each of 6 categories: RBC-low, RBC-medium, RBC-high, WBC-low, WBC-medium, and WBC-high. Each sample was analyzed 8-10 consecutive times (depending on the volume of urine available). A single sample could be used to fulfill 2 categories if it contained both RBC and WBC. Inter-assay precision was attempted using patient samples, but could not be completed due to cellular degradation over the 5-day period.

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[c] Moticam 5 digital microscopy camera, Motic®, China Group Co Ltd, Hong Kong, China
[d] IDEXX SediVue® QC fluid, IDEXX Laboratories Inc., Westbrook, ME

2.5 Statistical Analyses

All statistical analyses were performed using commercially available computer software.[e,f] The sensitivity and specificity with 95% confidence intervals of the SediVue (1.0.0.0 and 1.0.1.3) to detect clinically significant numbers of each formed element in comparison to manual microscopy were calculated. For these calculations, thresholds for clinical significance for both manual microscopy and SediVue analysis were defined as ≥ 5/HPF for RBC and WBC and ≥ 1 per HPF for EPI, NEC, TRI, and CaOxd. The following scale was used to rate sensitivity and specificity: excellent (95.0-100.0%), good (85.0-94.9%), moderate (70.0-84.9%), fair (60.0-69.9%), and poor (≤ 59.9%). In addition, receiver-operating characteristic (ROC) curve analysis was performed to determine the sensitivity and specificity of the SediVue for detection of each formed element at various thresholds, while holding the manual threshold constant. The SediVue thresholds with maximal sensitivity and specificity for each element were determined by calculating the Youden index (sensitivity + specificity – 1).

[e] Microsoft Excel 2013, Microsoft Corporation, Redmond, WA
[f] MedCalc Version 17.6, MedCalc Software bvba, Ostend, Belgium

3. RESULTS

3.1 Urine Samples

A total of 562 urine samples were evaluated. Of these, 26 samples were excluded from data analysis for the following reasons: problem with instrument hardware at time of analysis (n=13), sample mislabeling (n=5), excessive time between SediVue analysis and manual microscopic review (n=4), unknown volume of urine centrifuged (n=3), and excessive time between manual microscopic review by the two observers (n=1). This resulted in a total of 536 samples for study inclusion (435 canine and 101 feline). An additional 30 samples were excluded only for analysis of EPI and NEC due to numerical rounding during manual microscopic examination. The number of urine samples positive for each element on manual microscopy are listed in Table 2. The volume of urine centrifuged was between 1.1-1.4 mL for 3% of samples, 1.5-1.9 mL for 8% of samples, 2.0-2.4 mL for 13% of samples, 2.5-2.9 mL centrifuged for 18% of samples, 3.0 mL for 57% of samples, and 3.1-4.5 for 1% of samples.

Table 2. Number of urine samples positive for each element on manual microscopy.

	Total	**Canine**	**Feline**	**Positive threshold**
RBC	174/536	122/435	52/101	≥ 5/HPF
WBC	129/536	116/435	13/101	≥ 5/HPF
EPI	24/506	23/411	1/95	≥ 1/HPF
NEC	58/506	51/411	7/95	≥ 1/HPF
TRI	127/536	102/435	25/101	≥ 1/HPF
CaOxd	52/536	49/435	3/101	≥ 1/HPF

RBC, red blood cells; WBC, white blood cells; EPI, squamous epithelial cells; NEC, non-squamous epithelial cells; TRI, struvite crystals; CaOxd, calcium oxalate dihydrate crystals; HPF, high power field.

3.2 Comparison of SediVue Detection of Formed Elements to Manual Microscopy Using Set Thresholds

The sensitivity and specificity of the SediVue (1.0.0.0 and 1.0.1.3) for detection of formed elements in urine are presented in Table 3. Overall, the sensitivity of the SediVue (1.0.1.3) was good for detection of RBC, WBC, and TRI; moderate for CaOxd; fair for NEC; and poor for EPI. For all elements, the sensitivity increased in the newer software version (1.0.1.3) as compared to the older version (1.0.0.0). The specificity of the SediVue (1.0.1.3) was excellent for detection of EPI and CaOxd; good for RBC, WBC, and NEC; and moderate for TRI. For all formed elements except EPI, specificity decreased in software version 1.0.1.3 as compared to 1.0.0.0. For EPI, specificity increased minimally.

Table 3. Sensitivities and specificities of the SediVue for detection of formed elements in urine in comparison to manual microscopy (using the thresholds listed in Table 2).

	SediVue 1.0.0.0		**SediVue 1.0.1.3**	
	Sensitivity (95% CI)	**Specificity (95% CI)**	**Sensitivity (95% CI)**	**Specificity (95% CI)**
RBC	84.5% (78.2-89.5%)	93.4% (90.3-95.7%)	89.1% (83.5-93.3%)	90.3% (86.8-93.2%)
WBC	76.0% (67.7-83.1%)	94.4% (91.6-96.4%)	85.3% (78.0-90.9%)	88.7% (85.2-91.6%)
EPI	25.0% (9.8-46.7%)	98.8% (97.3-99.6%)	33.3% (15.6-55.3%)	99.4% (98.2-99.9%)
NEC	55.2% (41.5-68.3%)	89.3% (86.1-92.0%)	69.0% (55.5-80.5%)	86.6% (83.1-89.6%)
TRI	71.7% (63.0-79.3%)	92.9% (90.0-95.2%)	90.6% (84.1-95.0%)	83.9% (79.9-87.3%)
CaOxd	63.5% (49.0-76.4%)	99.8% (98.9-100.0%)	75.0% (61.1-86.0%)	99.2% (97.9-99.8%)

CI, confidence intervals; RBC, red blood cells; WBC, white blood cells; EPI, squamous epithelial cells; NEC, non-squamous epithelial cells, TRI, struvite crystals, CaOxd, calcium oxalate dihydrate crystals.

3.3 Evaluation of False Negative and False Positive Results for Select Elements

Samples with false negative results for EPI, NEC, and CaOxd were carefully reviewed due to the lower sensitivities observed for these elements compared with the other elements.

Sixty-seven percent of samples that were positive for EPI on manual microscopy (16/24) had false negative results on SediVue analysis. For these samples, the average number of EPI detected by manual microscopy was 1.8/HPF (range 1.0 – 3.7/HPF), while the average number detected by SediVue analysis was 0.3/HPF (range 0 – 0.9/HPF). Mislabeling of EPI as NEC was noted as one possible cause of false negative results.

For NEC, thirty-one percent of samples that were positive on manual microscopy (18/58) had false negative results. These samples were frequently densely cellular, containing > 100 RBC and/or WBC/HPF. Other samples were crowded with large amounts of other elements such as bacteria, sperm, or amorphous crystals.

Twenty-five percent of samples that were positive for CaOxd on manual microscopy (13/52) had false negative results, for which several causes were identified. In some samples, CaOxd were extremely small. In other samples, CaOxd were recognized as crystals but labelled as CRYu or TRI by the SediVue.

The specificity for TRI was the lowest of all elements (84%), with 16% of samples that were negative for TRI on manual microscopy (66/409) displaying false positive results. Review of images from these samples revealed that the SediVue sometimes misclassified other crystal types (e.g. CaOxd, calcium oxalate monohydrate,

amorphous crystals, and ammonium biurates) as TRI. Other formed elements such as RBC, WBC, and NEC (particularly when viewed on an angle or in densely crowded samples) were also occasionally misclassified as TRI. Additionally, various types of debris/contaminants were frequently incorrectly labelled as TRI. Lastly, in several samples, TRI were present and correctly identified in SediVue images. However, on manual microscopic examination of these samples, only rare TRI were observed that were unevenly distributed and not present in high enough numbers per HPF to exceed the required threshold for a positive result.

3.4 ROC Analysis for Determination of Optimal Thresholds

The ROC curves for each element are displayed in Figures 2 and 3 for both software versions. The ability of the SediVue to detect EPI, NEC, TRI, and CaOxd improved notably in 1.0.1.3, while there appeared to be minimal improvement for detection of RBC and WBC. The SediVue (1.0.1.3) thresholds with optimal sensitivity and specificity for detection of each element are listed in Table 4. The optimal thresholds for RBC, WBC, NEC, and TRI were similar to the original thresholds used. In contrast, for EPI and CaOxd, there was a large difference between the optimal and original thresholds.

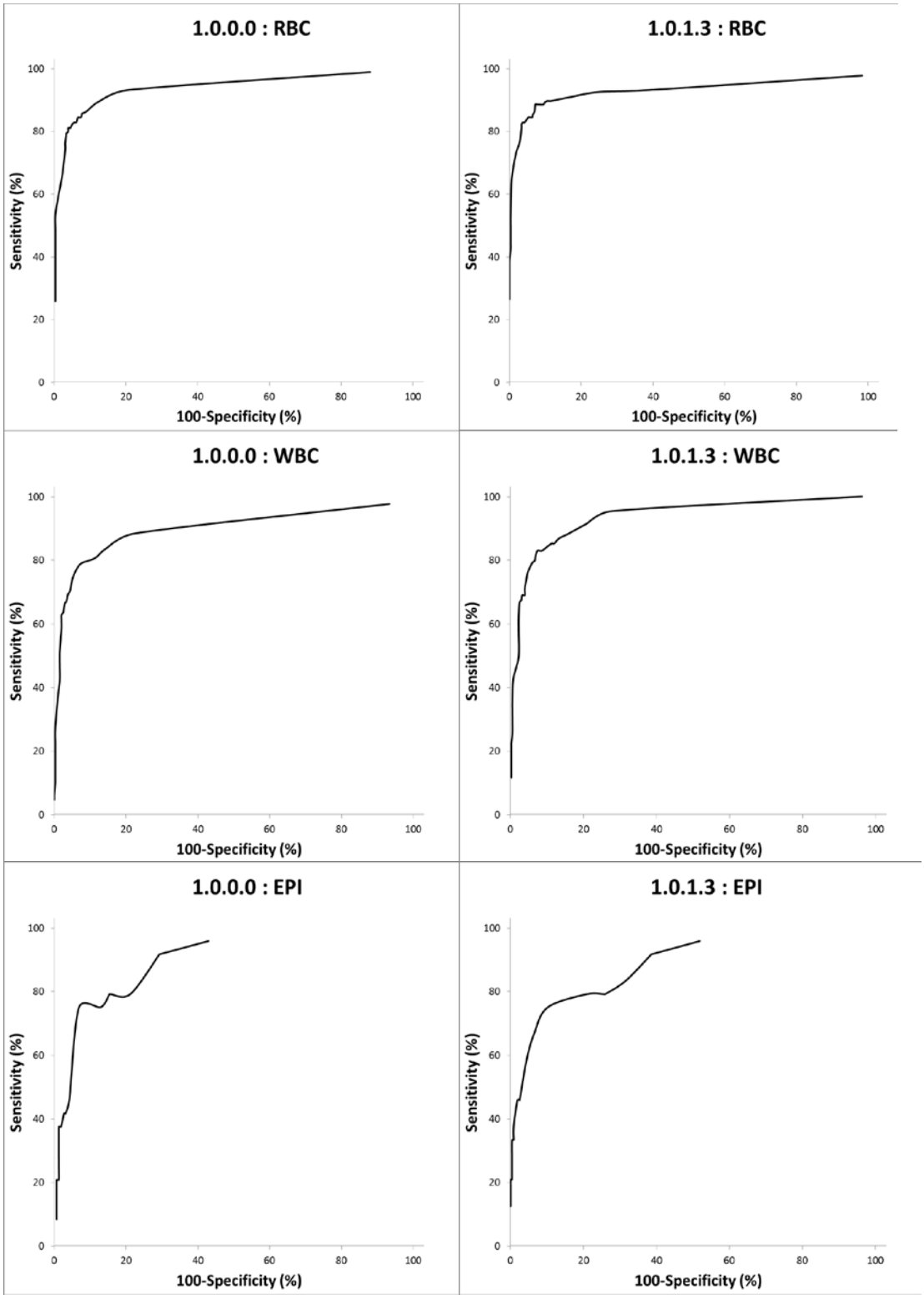

Figure 2. ROC curves for detection of RBC, WBC, and EPI by the SediVue (1.0.0.0 and 1.0.1.3).

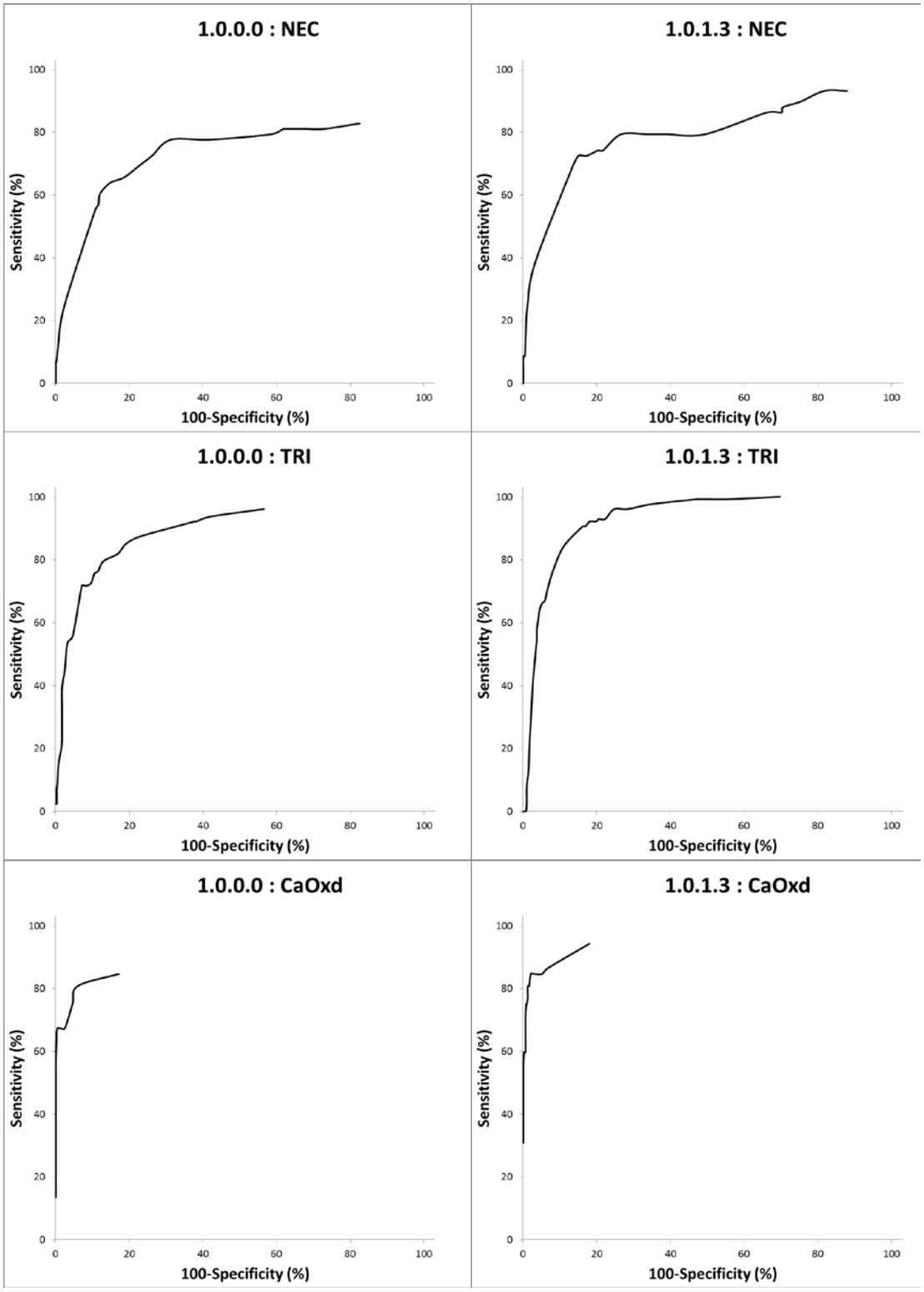

Figure 3. ROC curves for detection of NEC, TRI, and CaOxd by the SediVue (1.0.0.0 and 1.0.1.3).

Table 4. Optimal SediVue (1.0.1.3) thresholds for detection of formed elements in urine based on ROC analysis with corresponding sensitivities and specificities.

	Optimal threshold	Sensitivity (95% CI)	Specificity (95% CI)
RBC	6.5/HPF	88.5% (82.8-92.8%)	92.8% (89.7-95.3%)
WBC	6.5/HPF	83.0% (75.3-89.0%)	92.6% (89.6-95.0%)
EPI	0.2/HPF	75.0% (53.3-90.2%)	89.8% (86.8-92.4%)
NEC	0.9/HPF	72.4% (59.1-83.3%)	85.0% (81.4-88.2%)
TRI	1.0/HPF	90.6% (84.1-95.0%)	83.9% (79.9-87.3%)
CaOxd	0.3/HPF	84.6% (71.9-93.1%)	97.9% (96.2-99.0%)

CI, confidence intervals; RBC, red blood cells; WBC white blood cells; EPI, squamous epithelial cells; NEC, non-squamous epithelial cells; TRI, struvite crystals; CaOxd, calcium oxalate dihydrate crystals.

3.5 Comparison between Canine and Feline Samples

The sensitivity and specificity of the SediVue (1.0.1.3) for the detection of formed elements in canine versus feline urine samples are shown in Table 5. The sensitivity of the SediVue was similar for detection of RBC and TRI between canine and feline samples. In contrast, sensitivity for detection of WBC in feline samples was much lower than canine samples. The number of feline samples positive on manual microscopy for EPI, NEC, and CaOxd was too low ($n < 10$ for each element) to provide a valuable comparison of sensitivity between species. For all elements, specificity of the SediVue was similar between canine and feline samples.

Table 5. Sensitivities and specificities of the SediVue (1.0.1.3) for detection of formed elements in canine versus feline urine.

	Canine urine		Feline urine	
	Sensitivity (95% CI)	**Specificity (95% CI)**	**Sensitivity (95% CI)**	**Specificity (95% CI)**
RBC	87.7% (80.5-93.0%)	91.1% (87.3-94.0%)	92.3% (81.5-97.9%)	85.7% (72.8-94.0%)
WBC	87.9% (80.6-93.2%)	87.8% (83.7-91.2%)	61.5% (31.6-86.1%)	92.1% (84.3-96.7%)
EPI	30.4% (13.2-52.9%)	99.2% (97.8-99.8%)	100.0% (2.5-100.0%)	100.0% (96.2-100.0%)
NEC	68.6% (54.1-80.9%)	84.4% (80.3-88.0%)	71.4% (29.0-96.3%)	95.5% (88.8-98.8%)
TRI	90.2% (82.7-95.2%)	82.9% (78.4-86.8%)	92.0% (74.0-99.0%)	88.2% (78.7-94.4%)
CaOxd	79.6% (65.7-89.8%)	99.5% (98.1-99.9%)	0.0% (0.0-70.8%)	98.0% (92.8-99.8%)

CI, confidence intervals; RBC, red blood cells; WBC white blood cells; EPI, squamous epithelial cells; NEC, non-squamous epithelial cells; TRI, struvite crystals; CaOxd, calcium oxalate dihydrate crystals.

To understand the discrepancy in sensitivity for detection of WBC between species, feline samples with false negative results for WBC (n=5) were examined. Four of five samples contained > 100 RBC/HPF, while the final sample contained a large amount of amorphous crystals.

3.6 Comparison of Fresh and Stored Samples

Because the study contained a mixture of fresh (n=230) and stored samples (n=304), the diagnostic performance of the SediVue (1.0.1.3) in these categories of urine was compared. Fresh samples were analyzed the day of collection, while stored urine samples were analyzed > 1 day following collection. The sensitivities and specificities for fresh and stored samples are shown in Table 6. Overall, results for both groups were

similar, with the exception of higher sensitivity for TRI and higher specificity for RBC in stored urine. The number of fresh samples positive on manual microscopy for EPI and CaOxd was too low (n < 10 for each element) to provide a valuable comparison of sensitivities between categories.

Table 6. Sensitivity and specificity of the SediVue (1.0.1.3) for detection of formed elements in fresh versus stored urine.

	Fresh urine		**Stored urine**	
	Sensitivity (95% CI)	**Specificity (95% CI)**	**Sensitivity (95% CI)**	**Specificity (95% CI)**
RBC	89.5% (80.3-95.3%)	85.4% (78.8-90.5%)	88.8% (80.8-94.3%)	94.2% (90.1-97.0%)
WBC	87.8% (73.8-95.9%)	88.0% (82.6-92.3%)	83.2% (73.7-90.3%)	89.3% (84.4-93.1%)
EPI	0.0% (0.0-84.2%)	100.0% (98.2-100.0%)	36.4% (17.2-59.3%)	98.9% (96.9-99.8%)
NEC	65.0% (40.8-84.6%)	89.6% (84.3-93.6%)	69.2% (52.4-83.0%)	84.5% (79.6-88.7%)
TRI	75.0% (42.8-94.5%)	86.0% (80.7-90.3%)	92.2% (85.7-96.4%)	81.0% (74.6-86.3%)
CaOxd	0.0% (0.0-70.8%)	99.1% (96.9-99.9%)	79.6% (65.7-89.8%)	99.2% (97.2-99.9%)

CI, confidence intervals; RBC, red blood cells; WBC white blood cells; EPI, squamous epithelial cells; NEC, non-squamous epithelial cells; TRI, struvite crystals; CaOxd, calcium oxalate dihydrate crystals.

3.7 Intra- and Inter-assay Precision

Intra- and inter-assay precision data for QCM are shown in Table 7, and intra-assay precision data for patient samples are shown in Table 8. Precision was overall acceptable, with average coefficients of variation (CVs) for each category < 20%. Note that the CV could not be calculated for RBC in “normal” QCM, as the numbers of RBC present were too low (< 1/HPF).

Table 7. Intra- and inter-assay precision data for quality control material.

		Intra-assay		Inter-assay	
		Number of cells/HPF (mean and range)	**CV (%)**	**Number of cells/HPF (mean and range)**	**CV (%)**
RBC	**Normal**	N/A	N/A	N/A	N/A
	Abnormal	33.6 (30.8-35.9)	4.6	34.7 (30.8-37.1)	7.6
WBC	**Normal**	2.6 (1.9-3.3)	15.6	2.6 (2.6-2.7)	1.3
	Abnormal	32.1 (27.7-39.5)	10.6	33.0 (29.1-35.0)	7.1

HPF, high power field; CV, coefficient of variation; RBC, red blood cells; WBC, white blood cells.

Table 8. Intra-assay precision data for patient samples.

		Number of samples	**Number of cells/HPF (mean and range)**	**CV (%, mean and range)**
RBC	**Low**	5	2.9 (1.1-5.4)	19.3 (11.9-25.4)
	Medium	4	13.2 (5.6-19.2)	9.9 (6.9-14.7)
	High	1	26.4 (24.9-28.1)	4.5
WBC	**Low**	3	2.1 (0.8-3.8)	10.6 (7.9-12.0)
	Medium	6	11.6 (5.5-18.6)	11.9 (8.0-16.6)
	High	3	41.7 (28.6-54.4)	10.3 (7.3-15.9)

HPF, high power field; CV, coefficient of variation; RBC, red blood cells; WBC, white blood cells.

4. DISCUSSION

The recent introduction of the IDEXX SediVue Dx® Urine Sediment Analyzer provides veterinary personnel with a much anticipated automated method for urine sediment analysis. This is the first study to evaluate the performance of the SediVue for detection of cells and crystals in urine compared to manual microscopy. Overall, the ability of the SediVue (1.0.1.3) to detect clinically significant numbers of formed elements is good for RBC, WBC, TRI, and CaOxd, while improvement is needed for accurate detection of epithelial cells.

The SediVue exhibited good sensitivity for detection of RBC, WBC, and TRI, with lower sensitivity for EPI, NEC, and CaOxd. In particular, the sensitivity for detection of EPI was poor (33%). This poor sensitivity could be partially due to the low number of samples positive for EPI on manual microscopy. While many samples contained scattered EPI, only 5% of samples contained enough EPI to exceed the positive threshold of ≥ 1/HPF, resulting in a wide 95% confidence interval for sensitivity. In the future, evaluation of additional samples containing EPI would result in a narrower confidence interval and provide a more accurate reflection of the true sensitivity. Regardless, the upper confidence limit of 55% is still poor, supporting the need for improvement in EPI detection. It is important to note, however, that although ≥ 1 EPI/HPF was considered significant for the purpose of this study, a positive result for EPI is not necessarily indicative of a pathologic process. Squamous epithelial cells are

normally found lining the distal urinary tract, and their presence in urine sediments is generally regarded as an incidental finding.

To help understand why the SediVue had low sensitivity for EPI detection and investigate whether this could have clinically significant consequences, samples with a false negative result for EPI were reviewed. The average number of EPI detected was 1.8/HPF for manual microscopy versus 0.3/HPF for SediVue analysis. The clinical significance of this finding appears minimal, as the difference between 1.8 and 0.3 EPI/HPF would have no impact on clinical decision-making. However, several of these samples also had a false positive result for NEC, and review of the SediVue images for these samples confirmed mislabeling of EPI as NEC in some samples. This could potentially have a clinical impact, as misclassification of EPI as NEC may raise unnecessary suspicion for a pathologic process such as transitional cell carcinoma. Therefore, the distinction between types of epithelial cells by the SediVue represents an area for future improvement. However, this issue is not unique to SediVue analysis, as epithelial cell types can be difficult to distinguish even for human observers. In fact, some reference laboratories do not routinely distinguish between EPI and NEC, simply reporting both types as "epithelial cells."

With regard to the relatively low sensitivity for other elements, a variety of reasons were found. For NEC, the low sensitivity was partially due to overshadowing of relatively low numbers of NEC by large numbers of WBC and RBC in active sediments. For CaOxd, several of the false negative samples contained CaOxd that were extremely small and difficult to distinguish from background debris on manual microscopy, which

is likely the reason that these crystals were missed by the SediVue. These structures were suspected to be CaOxd on manual microscopy due to their refractile nature and subtle diamond shape, occasionally with a faintly visible “x” across the center. CaOxd origin was supported by solubility testing, as the crystals were insoluble in acetic acid but soluble in hydrochloric acid. In addition, for several of the other false negative samples, CaOxd were labelled as CRYu by the SediVue. Therefore, the SediVue was able to identify that there were crystals present, but did not specifically label the crystals as CaOxd. Mislabeling of CaOxd as TRI was also noted in several samples (discussed further below).

As a whole, the specificity of the SediVue for detection of formed elements was good to excellent. The lowest specificity observed was for detection of TRI. In some samples with false positive results, scattered TRI were observed on manual microscopic examination, although not in high enough numbers to meet the requirements for a positive result. Therefore, this study may underestimate the specificity of the SediVue for TRI detection. The fact that more TRI were observed per HPF on SediVue analysis compared to manual microscopy may suggest increased sedimentation of formed elements by the SediVue or more even distribution of elements in SediVue preparations. Another cause of false positive results was mislabeling of other crystal types as TRI, supporting that improvement is needed for distinction between TRI and other crystal types. Additionally, the incorrect labeling of various forms of debris and environmental contaminants as TRI suggests the need for continued enhancement in specificity for TRI detection.

During the course of this study, several software iterations were developed to improve detection of formed elements, and the latest of these versions (1.0.1.3) was compared with one of the earliest versions (1.0.0.0). The sensitivity of the SediVue for the detection of all formed elements increased with the later version, at the expense of a typically mild decrease in specificity. This demonstrates the ability of the SediVue to improve over time with adjustments to the neural-network algorithm, and the software will continue to be refined to enhance detection of cells and crystals, with particular focus on improving sensitivity for elements such as EPI, NEC, and CaOxd.

Comparison of ROC curves between 1.0.0.0 and 1.0.1.3 illustrates improvement in overall SediVue performance in the later software version, although the degree of improvement varied depending on the element. Improvement in detection of EPI, NEC, TRI, and CaOxd was evident, while detection of RBC and WBC improved minimally. This is due to the fact that detection of RBC and WBC in 1.0.0.0 was already satisfactory; therefore, the focus for 1.0.1.3 was improvement in epithelial cell and crystal detection. Unfortunately, areas under the curve (AUC) could not be calculated because the ROC curves did not extend to 100 on the x-axis, due to the fact that the number of false negative results never reached 0, even at a threshold of 0 elements/HPF.

ROC analysis was utilized to determine optimal thresholds for detection of each element when compared with the originally-defined thresholds for manual microscopy. While the optimal thresholds were similar to the original thresholds for most elements, the optimal thresholds for EPI and CaOxd were notably lower. For both elements, utilizing the optimal threshold resulted in a substantial increase in sensitivity, albeit at

the expense of a mild to moderate decrease in specificity. In the future, it is possible that these thresholds may be incorporated into the neural-network algorithm to improve detection of formed elements.

Sensitivity and specificity of the SediVue for detection of formed elements were generally similar between canine and feline samples, with the exception of sensitivity for WBC. The lower sensitivity for detection of WBC in feline samples compared to canine samples could be at least partially due to the low number of feline samples that contained ≥ 5 WBC/HPF on manual microscopy (n=13). In general, UTIs are uncommon in feline patients, likely explaining the low number of positive samples on manual microscopy, and this resulted in a wide confidence interval for sensitivity. In addition, the feline samples with false-negative results for WBC were densely crowded with large numbers of RBC or amorphous crystals, masking the relatively low but still significant number of WBC present. In the future, collection of additional pyuric feline samples will be useful to narrow the confidence interval and more accurately determine sensitivity. In addition, dilution of specimens may improve detection of WBC and other formed elements in densely crowded samples.

The urine samples used in this study included a mixture of fresh and stored samples. To look for a possible impact of the age of urine on SediVue performance, the sensitivities and specificities for the detection of formed elements in fresh and stored samples were compared. The sensitivities and specificities were similar for most elements, with the exception of specificity for RBC and sensitivity for TRI, both of which were higher in stored samples. For TRI, a possible cause for the discrepancy in

sensitivity is the number of samples positive in each category. The majority of samples that were positive for TRI on manual microscopy had been stored prior to analysis (n=115), while a much lower number of fresh samples were positive on manual microscopy (n=12). For this reason, the 95% confidence interval for sensitivity for TRI detection was wide for fresh samples, and it is possible that the sensitivity in stored samples is a better reflection of the true sensitivity for TRI detection. The reason for the higher specificity for RBC in stored samples is unclear, but could be at least partially due to the larger number of stored samples that were negative for RBC on manual microscopy (n=206 for stored samples versus n=157 for fresh samples).

The SediVue displayed very good precision for detection of RBC and WBC, especially compared to that reported for manual microscopy. In a number of studies, the imprecision of manual microscopy is consistently higher than that reported for automated methods. For manual microscopy, CVs as low as 8.5% have been reported for samples with large numbers of cells; however, CVs typically exceed 40% in lowly cellular samples.[8,20,29-31,34] In the current study, imprecision of the SediVue tended to be greater in samples with low cell counts, with a few exceptions (e.g., inter-assay CV of 1.3% for WBC in QCM containing < 5 WBC/HPF). Regarding intra-assay precision, CVs obtained using QCM and patient samples were comparable. This suggests sufficient repeatability of results for patient samples that are re-analyzed within a short period of time (i.e., one hour). However, the same cannot be said for samples that are re-analyzed within one or several days. Urine is a very unstable environment, with cells and other formed elements degenerating at unpredictable but typically rapid rates. While some

elements degrade with time, others may actually proliferate (specifically crystals, bacteria, and yeast) and obscure other elements. Calculation of inter-assay precision was attempted for patient samples (Appendix A). However, as expected, results were extremely variable. In general, WBC numbers decreased over time, while RBC numbers tended to increase over time. In several samples, examination of SediVue images on subsequent days after initial analysis revealed misclassification of WBC as RBC, providing at least a partial explanation for the trends described above. These misclassified WBC were often smaller than the majority of WBC.

While not always as effective as traditional microscopy for identification of formed elements, the SediVue offers numerous advantages over manual microscopic examination of urine sediments. Microscopic examination of urine sediments is time-consuming, and many veterinarians and technicians do not feel comfortable identifying formed elements in urine samples. Additionally, manual preparation of urine sediments is poorly standardized. Variables such as the amount of urine centrifuged, force of centrifugation, and method used for sediment examination (e.g., slide-and-coverslip versus DeciSlide™) vary greatly between clinics. Within a single clinic, there may be significant inter-operator variation regarding urine sediment preparation, including different methods for decanting the supernatant. Even if a single person performs every sediment analysis, there may be variation in the amount of supernatant used for resuspension of sediment, or the volume of sediment placed on the slide for microscopic examination. Manual microscopic examination of urine is also associated with high inter-observer variability with regard to identification and quantification of formed

elements, further contributing to the imprecision associated with this method.[1-3] Lastly, standard centrifugation of urine may destroy fragile formed elements, potentially providing an inaccurate representation of the true contents of the sample.[2]

The introduction of the SediVue Dx for automation of urine sediment analysis addresses many of the aforementioned issues. In contrast to manual microscopy, the SediVue utilizes a very small volume of uncentrifuged urine, minimizing the amount of urine required for complete urinalysis. Because urine does not need to be centrifuged prior to SediVue analysis, the number of steps involved in sediment preparation is reduced, eliminating several of the previously discussed sources of variation. In addition, the volume of urine and force of centrifugation are standardized. The SediVue utilizes a short and gentle centrifugation technique, which could reduce destruction of fragile formed elements. In addition, the results of this study indicate that the SediVue exhibits improved precision for detection and quantification of formed elements in urine sediments in comparison to what is reported for human observers. Importantly, the ability of the SediVue to capture high-resolution images of urine sediment is a major benefit. Images are displayed for review by the operator, minimizing the need for veterinarians and technicians to examine urine manually under the microscope and reducing the time and effort required for sediment review. Additionally, the images may be incorporated into the medical record or shared with experts in the field for consultation on difficult samples.

Despite these advantages, there are several limitations associated with this instrument. The SediVue may misclassify some elements, particularly those that are also

challenging for human observers. Difficulties distinguishing EPI and NEC have already been discussed. Additionally, although not evaluated in this study, identification of casts and bacterial cocci are notoriously difficult for human observers on wet preparations of urine sediment and are currently areas needing improvement for the SediVue. Differentiating bacterial cocci from particulate debris or amorphous crystals can be extremely difficult without confirmation using a stained slide or solubility testing, respectively. Casts can be difficult to distinguish from mucus strands, fibers, or other cylindrical contaminants. Future versions of the neural-network algorithm should continue to enhance the ability of the SediVue to identify these elements.

In addition, samples that are densely cellular will likely need to be diluted in order for the SediVue to accurately identify and quantify formed elements. Dilutions were not performed in this study for consistency in the experimental protocol. Therefore, results from densely cellular samples were often inaccurate and likely had a negative impact on the reported sensitivities and specificities (Figure 4). In the commercial version of the SediVue, however, results are not reported if a sample is too crowded for the SediVue to accurately identify and quantify formed elements. Instead, the run is flagged, and the instrument prompts the operator to dilute the sample. Customers are provided with a protocol containing reference images from samples with varying degrees of “crowding.” Based on the density of cells or other elements, sample dilution is recommended using a particular volume of saline. Additional studies could be useful to assess the degree of improvement in formed element detection upon dilution of densely cellular samples.

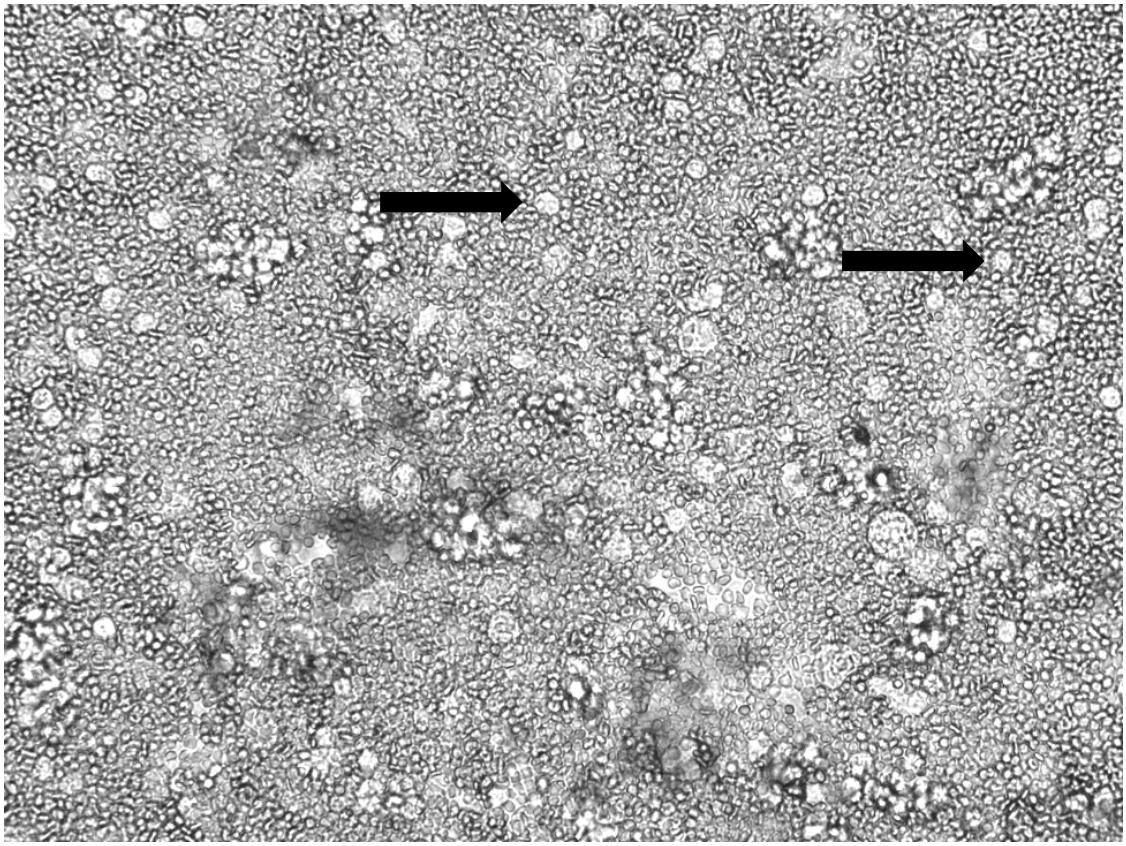

Figure 4. SediVue image from a densely cellular urine sample. The vast majority of cells are RBC, with fewer but significant numbers of WBC (arrows). The SediVue displayed false negative results for both RBC and WBC due to the extremely crowded nature of the sample.

Another limitation is the impact of lipid droplets on SediVue performance, as the instrument often had difficulty in our study focusing on samples with large numbers of lipids. Lipid droplets are common in urine samples from veterinary species. Because lipids tend to reside in the plane above other formed elements, the SediVue camera sometimes focuses on the lipid plane rather than the cellular plane, producing images that are out of focus (Figure 5). This is mainly a problem in lowly cellular samples with large numbers of lipid droplets. To address this problem, the focusing and centrifugation protocols of the SediVue have recently been modified to reduce interference from lipids.

Specifically, increasing the centrifugation time from 10 seconds to 30 seconds enhances separation of lipids from other formed elements, allowing the SediVue to more easily focus on cells and crystals rather than lipids. However, for consistency, the centrifugation time was maintained at 10 seconds for all samples in this study.

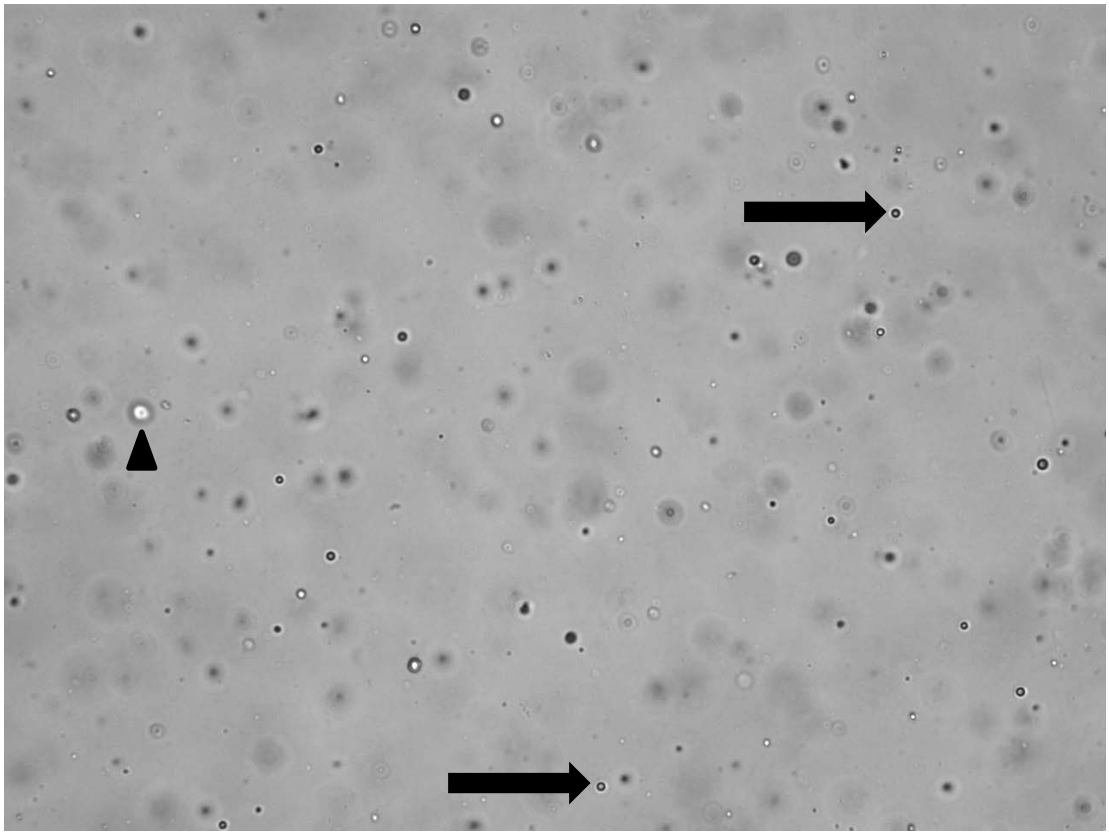

Figure 5. SediVue image from a lowly cellular sample with lipid droplets (arrows). The arrowhead indicates a possible cell, but its identity cannot be determined because the image is focused in the lipid plane.

Practitioners should be keenly aware of the necessity of mixing urine samples immediately prior to SediVue analysis. Formed elements in urine samples settle rapidly after mixing.[11] In fact, settling of elements may occur in as little as 15 seconds.[35] Therefore, all samples for SediVue analysis should be thoroughly mixed and quickly

pipetted into the instrument, as settling of formed elements may have a major impact on results of SediVue analysis. For example, if a sample is not well mixed and formed elements settle to the bottom of the tube, collection of urine from the top of the sample would underestimate the number of cells and other elements. Conversely, collection from the bottom of the sample would overestimate the number of formed elements.

There were several limitations associated with this study, including the use of manual microscopy as a reference method for evaluation of SediVue performance. Although it is considered the gold standard for evaluation of urine sediments, there are many shortcomings associated with manual microscopy, as previously discussed. In an attempt to minimize the degree of inter-operator variation with regard to identification and quantification of formed elements, two observers evaluated each sediment and were required to reach a majority decision regarding the type and number of elements present. However, minor differences in operator performance, including the steps involved in urine sediment preparation, cannot be completely excluded as a cause for variation. Additionally, definitive identification of elements in urine sediments can be challenging for even experienced technicians and pathologists. For example, differentiation between RBC and WBC can be difficult in older samples as cells begin to degrade. Therefore, although traditionally considered the gold standard for sediment evaluation, manual microscopy is by no means an infallible method for identification of formed elements in urine samples.

In this study, a single method for urine sediment preparation and evaluation was used, limiting the ability to apply results to other laboratories. KOVA® tubes and

DeciSlides™ were selected since these are the materials standardly used by our clinical pathology laboratory for routine processing of urine samples. However, many reference laboratories and clinics use other preparation methods (e.g., a slide and coverslip) for microscopic examination. In the authors' experience, the same urine sediment, when examined using both the DeciSlide™ and slide-and-coverslip method, will appear significantly more concentrated on the DeciSlide™ preparation. Therefore, the sediment preparation method used in this study as the reference method to evaluate SediVue performance is not entirely reflective of the various methods utilized by veterinarians in practice.

The volume of urine centrifuged was not standardized in this study in order to maximize the number of samples available for analysis. Ideally, a standard volume of urine should be centrifuged for every urine sample to ensure consistency in comparison of results between samples. However, it is not always feasible to obtain a consistent amount of urine from every patient. Therefore, a minimum of 1.1 mL of urine was required for study inclusion, although the majority of samples (88%) had between 2.0 and 3.0 mL available for centrifugation. While ideally the volume of urine centrifuged should be standardized within a veterinary practice, in the authors' experience, the volume of urine centrifuged does not have a major impact on results of sediment examination, particularly when comparing such small differences in centrifuged volumes.[34]

While a large number of urine samples were included in this study, the number of samples positive on manual microscopy for certain elements was low. In particular, a

low proportion of samples were positive for EPI, NEC, and CaOxd. Therefore, the 95% confidence intervals for sensitivity for detection of these elements were wide. This was especially a problem for feline samples, as they composed a minority of samples. In the future, collection of additional samples containing significant numbers of EPI, NEC, and CaOxd would be useful to more accurately determine the sensitivity of the SediVue for detection of these elements. Collection of additional feline samples would also allow better characterization of SediVue performance in this species. Of note, given the relatively large number of samples that were considered negative for each element, the 95% confidence intervals for specificity are generally much narrower and, therefore, provide a more representative estimate of the specificity of the SediVue.

Lastly, while this study did evaluate the ability of the SediVue to accurately detect the presence or absence of clinically significant numbers of cells and crystals, the actual correlation between the number of elements identified per HPF using the SediVue as compared to manual microscopy was not evaluated. As an example, a sample with 10 RBC/HPF according to manual microscopic examination could have contained 50 RBC/HPF according to SediVue analysis. This would be considered a true positive result, as both methods exceeded the positive threshold of $\geq$ 5/HPF, despite the lack of close correlation between numerical results. In the future, the data collected in this study could be used to more precisely correlate the results of SediVue analysis with those of manual microscopy. However, such a correlation may not be transferrable to other laboratories given the variety of methods used to examine urine sediments manually that,

in the authors' experience, often do not correlate (e.g., slide-and-coverslip versus DeciSlide™ versus examination of uncentrifuged urine using inverted microscopy).[34]

5. SUMMARY AND CONCLUSIONS

In summary, while the SediVue does not entirely eliminate the need for veterinary personnel to visually evaluate urine sediments, it does provide diagnostically useful information and should increase the efficiency and ease of performing complete urinalyses in private practice. Future studies are planned to characterize the ability of the SediVue to detect casts, bacteria, and other types of crystals. Additionally, analysis of the performance of the SediVue in samples from other veterinary species could be useful. In particular, the SediVue would be of major benefit for evaluation of urine in rodents and exotic species, given the small volume of urine required for SediVue analysis. Lastly, further updates to the neural-network algorithm should continue to enhance accuracy of detection of formed elements in future software versions.

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APPENDIX A

PATIENT INTER-ASSAY PRECISION DATA

Table A-1. Inter-assay precision for RBC in patient samples.

Patient	Day	Number of cells/HPF	CV (%)
1	1	4.5	40.2
	2	10.5	
	3	10.5	
	4	16.5	
	5	13.7	
2	1	1.2	87.6
	2	4.4	
	3	15.8	
	4	20.3	
3	1	1.6	107.3
	2	6.7	
	3	12.9	
	4	36.6	
4	1	2.1	29.9
	2	4.3	
	3	3.8	
	4	4.5	
5	1	2.4	38.1
	2	1.6	
	3	1.2	

Table A-2. Inter-assay precision for WBC in patient samples. The first 5 samples correspond to the 5 samples presented in Table A-1.

Patient	Day	Number of cells/HPF	CV (%)
1	1	18.7	98.1
	2	9.5	
	3	4.0	
	4	2.2	
	5	1.8	
2	1	28.6	34.2
	2	34.8	
	3	20.7	
	4	15.5	
3	1	30.8	34.0
	2	77.7	
	3	67.2	
	4	61.7	
4	1	9.3	55.7
	2	4.6	
	3	3.5	
	4	3.1	
5	1	6.5	25.2
	2	5.1	
	3	3.9	
6	1	3.2	23.6
	2	3.9	
	3	2.6	
	4	2.3	
7	1	1.8	51.0
	2	1.0	
	3	0.7	